Plankton Analysis by Automated Submersible Imaging Flow Cytometry: Transforming a Specialized Research Instrument into a Broadly Accessible Tool and Extending its Target Size Range

Robert J. Olson
Woods Hole Oceanographic Institution, MS 32
Woods Hole, MA 02543
Phone: (508) 289-2565 FAX: (508) 457-2134 E-mail: rolson@whoi.edu

Heidi M. Sosik
Woods Hole Oceanographic Institution, MS 32
Woods Hole, MA 02543
Phone: (508) 289-2311 FAX: (508) 457-2134 E-mail: hsosik@whoi.edu

Award #: N000140811044
http://www.whoi.edu/sites/hsosik/

LONG TERM GOALS

Detailed knowledge of the composition and characteristics of the particles suspended in seawater is crucial to an understanding of the biology, optics and geochemistry of the oceans. The composition and size distribution of the phytoplankton community, for example, help determine the flow of carbon and nutrients through an ecosystem and can be important indicators of how coastal environments respond to anthropogenic disturbances such as nutrient loading and pollution. Our goal is to provide researchers with instruments to continuously monitor phytoplankton community structure and investigate questions about the world's ocean ecosystems.

OBJECTIVES

Flow cytometry is one of the most promising technologies for studies of the microscopic constituents of marine ecosystems (Moore et al. 2009; Sosik et al. 2009). The intent of this project is twofold: to commercialize a field-proven state-of-the-art submersible imaging flow cytometer for nano- and microplankton so that other researchers can utilize this exciting new technology, and to develop a next generation of the instrument with extended measurement range, capable of analyzing cells from pico- to microplankton.

APPROACH

We are developing a prototype commercial version of Imaging FlowCytobot (IFCB), reproducing its functions via a series of modular components whose integration will result in a simple and robust instrument that is both reliable and easy to manufacture. The first step involved a ground-up examination of an existing benchtop version of Imaging FlowCytobot. This examination established design goals for each functional module of the instrument (e.g., flow system, cell detector, imaging

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 2011		**00-00-2011 to 00-00-2011**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Plankton Analysis by Automated Submersible Imaging Flow Cytometry: Transforming a Specialized Research Instrument into a Broadly Accessible Tool and Extending its Target Size Range	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Woods Hole Oceanographic Institution,Woods Hole,MA,02543**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**7**	

system, signal processing electronics, control system). The redesign process began with a mechanical backbone analogous to the optical breadboard now used, onto which have been designed core functional modules for cell detection and imaging, to establish a working imaging system that utilizes electronics and fluidics analogous to those in the present Imaging FlowCytobot. This approach enables us to compare performance of the commercial prototype to that of the original instrument, and to identify problems with components or integration (such as incorrect physical layout or optical components). We completed the first beta unit of the new design in July (Figs. 1, 2), and tested it during a several-week deployment off the WHOI dock. The results of this test were satisfactory in terms of image quality (Fig. 3) and general functioning of the instrument; several electronic and software faults were identified and have been corrected.

This project was conceived and funded as a collaboration between the WHOI researchers and Cytopeia, Inc., a well-respected manufacturer of high-speed sorting flow cytometers. Although the market for Cytopeia's products was the biomedical community, the founder/president had long been interested in marine applications of flow cytometry, and had sold several slightly-modified instruments (called Influx Marina) to oceanographic institutions for phytoplankton research. Cytopeia expressed interest in a partnership to develop IFCB into a commercially viable product (i.e., smaller, more robust, user-friendly, and manufacturable), and offered to contribute gratis a significant amount of engineering time and machining to the redesign effort.

However, soon after the project's start, Cytopeia was acquired by a much larger company, BD Biosciences, and it gradually became clear that under the new corporate structure IFCB redesign was not a high priority. In the 2nd year of the project, after many delays and relatively little contribution from the Cytopeia (now BD) engineers, we were told that only stock Influx parts could be used in the new IFCB. This development made it clear that the project had to take a new tack to succeed, so we began searching for a new commercial partner, while continuing our own redesign efforts at WHOI. We made good progress by utilizing smaller, more efficient components (including a GigE camera, Atom-based computer, and modular flash lamp), designing our own opto-mechanical system and compact syringe pump, and working with an outside consultant to implement new electronics and software for instrument control and data acquisition. The current IFCB is less than half the weight and uses 1/3 the power of the research prototype, and is more stable and simpler to use. The basic redesign has met our targets for size and power consumption; manufacturability remains to be established.

We approached McLane Research, Inc. as a potential commercial partner at the beginning of this year. We knew of McLane through our WHOI Biology Department colleague Don Anderson, who was funded by NSF to purchase several Environmental Sample Processors (ESP), to be manufactured by McLane. The ESP was developed at MBARI by C. Scholin, in a process somewhat similar to ours with IFCB, and it is at least as complex as IFCB, so we watched with interest as McLane successfully manufactured these units. WHOI is now entering into a formal licensing agreement with McLane to build IFCBs, and we have begun the transfer of technology by having McLane engineers observing Olson during the construction of the next redesigned IFCB beta unit.

WORK COMPLETED

We have essentially achieved our design goals (the new instrument has already been deployed from the WHOI pier for several weeks of testing) by utilizing smaller, more efficient components: 1) We replaced the camera and its 2-board framegrabber by a new, smaller GigE camera which requires no framegrabber; 2) We replaced the PC104+ computer by a new model based on the Intel Atom processor, which uses 1/3 the power of the old computer; 3) we replaced the flash lamp module and its power supply by a new model that is smaller, takes less power, and requires no separate power supply. We also designed our own opto-mechanical system and compact syringe pump, and (working with an outside consultant) have implemented custom electronics for instrument control and data acquisition that reduce the number of boards in the system. The same consultant has supplied new software. The current IFCB is half as heavy and uses 1/3 the power of the research instrument, and is more stable and simpler to use. The basic redesign has thus met our targets for instrument size and power consumption; optimizing for manufacturability in now underway with our commercial partner, McLane Research, Inc. We have begun to familiarize McLane engineers with the instrument by having them observe the construction by Olson of the next IFCB beta unit.

RESULTS

We have completed the first beta unit (including a test deployment off the WHOI dock for several weeks). This design incorporates a compact and rigid optical structure, a new prototype syringe pump/distribution valve system, a new energy efficient computer and camera, and newly designed data acquisition and instrument control electronics. These changes combine to reduce the instrument's size and power requirement significantly, which will enhance its utility for non-cabled platforms. We have also obtained improved syringes and implemented and tested a dual gear pump system that will allow longer deployments, increase reliability, and reduce maintenance costs.

IMPACT/APPLICATIONS

National Security

There is potential for this application to be useful for detecting pathogens in water supplies.

Economic Development

The Imaging FlowCytobot represents a potential new product line, since it has utility for plankton ecologists studying plankton processes (including effects of pollution and climate and change), and also for water resource managers (as a means to monitor harmful algal species).

Quality of Life

Species-level information is critical for such societally important problems as understanding the regulation and fate of regional harmful algal blooms. At the global scale, it is becoming increasingly evident that simple nutrient-phytoplankton-zooplankton models are inadequate for predicting effects of environmental change and that biogeochemical functional groups such as nitrogen fixers, silicifiers, and calcifiers need be resolved. We presently lack observational capabilities to provide data for

building and evaluating models, as well as for developing new approaches such as satellite-based remote sensing approaches to monitor functional group distributions. Widespread availability of instruments such as Imaging FlowCytobot will be an important step to overcoming present observational limitations.

Science Education and Communication

The images of individual plankton cells provided by these instruments, remotely and in near-real time, should contribute effective components of educational programs about the oceans, both in science curricula and for the general public.

TRANSITIONS

Quality of Life

A prototype Imaging FlowCytobot has already provided early warning of a toxic dinoflagellate blooms in the Gulf of Mexico (the first toxic *Dinophysis* bloom observed in Texas waters, and subsequent *Karenia brevis* blooms), allowing timely closure of shellfisheries that prevented human illnesses.

Science Education and Communication

Images from a prototype Imaging FlowCytobot have been circulated to plankton experts via the Internet, allowing species identification and better interpretation of potential processes behind bloom dynamics.

RELATED PROJECTS

This project builds on previous projects in the Olson and Sosik laboratories See http://www.whoi.edu/sites/hsosik/ for more details.

PUBLICATIONS

Moore, C., A. Barnard, P. Fietzek, M. R. Lewis, H. M. Sosik, S. White, and O. Zielinski. Optical tools for ocean monitoring and research. 2009. Ocean Science. 5, 661–684.

Sosik, H. M. 2008. Characterizing seawater constituents from optical properties. In M. Babin, C. S. Roesler and J. J. Cullen [eds.], Real-time coastal observing systems for ecosystem dynamics and harmful algal blooms: Theory, instrumentation and modelling. UNESCO, p. 281-329.

Sosik, H. M., R. J. Olson, and E. V. Armbrust. 2010. Flow cytometry in plankton research. *In* D. J. Suggett, O. Prasil and M. A. Borowitzka [eds.], Chlorophyll a fluorescence in aquatic sciences: methods and applications. Springer.

The original IFCB (left) requires a 12” diameter housing and weighs 200 lb. The first completed beta unit (right) is much smaller (8” diam) and light enough to lift by hand.

Fig. 1. Original IFCB and first beta unit

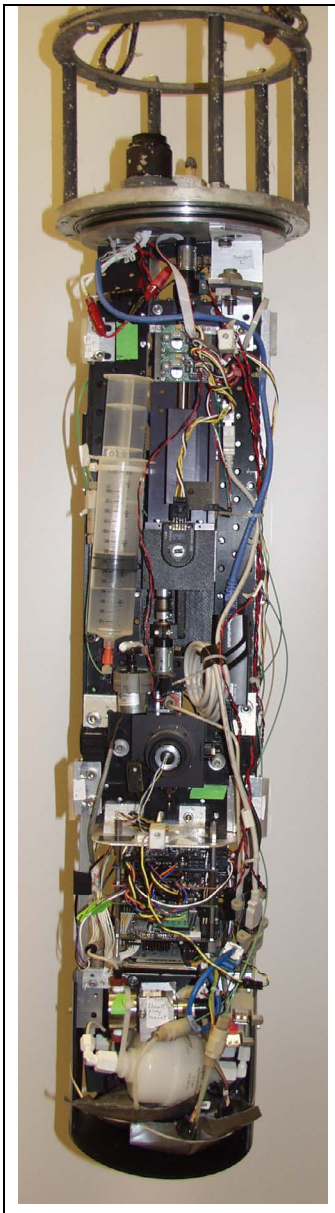	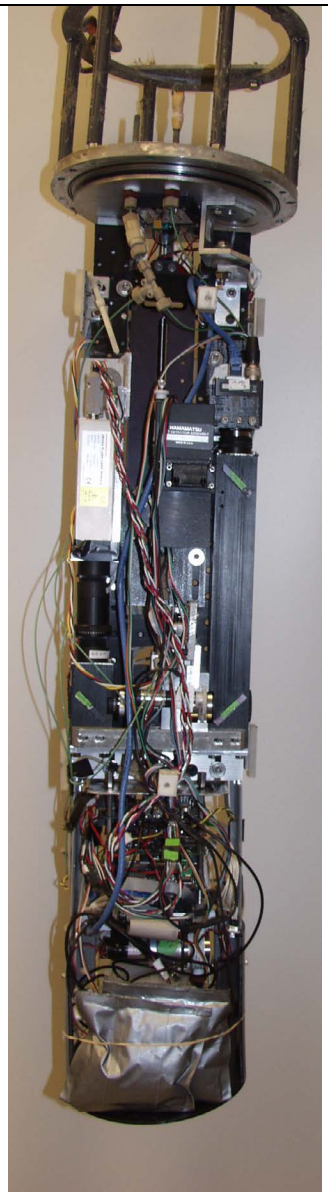	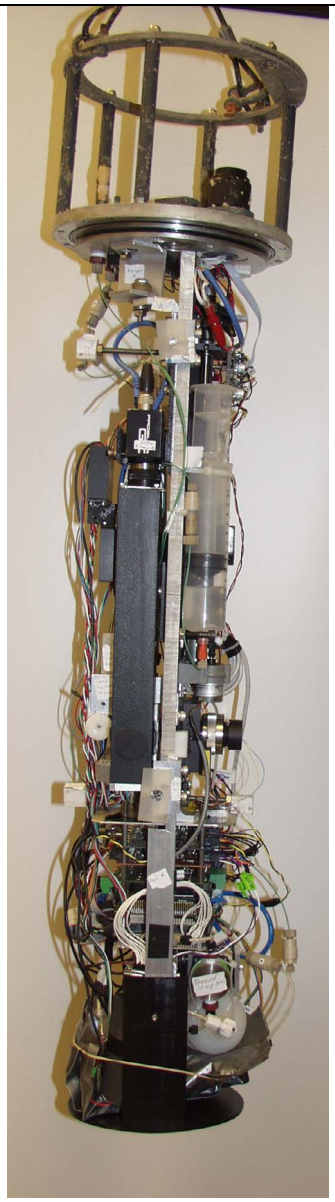	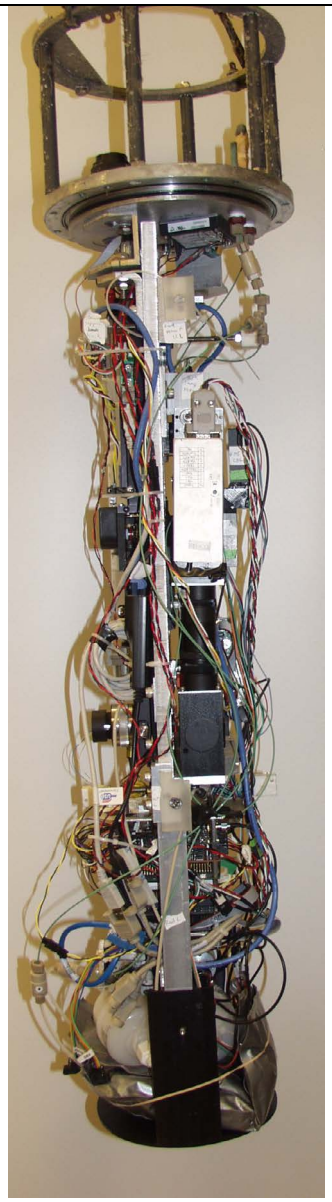
Front (laser, beads)	Back (optics, flow cell)	Left (detectors, beads)	Right (flash lamp)

Fig. 2. First beta unit (out of its watertight housing)

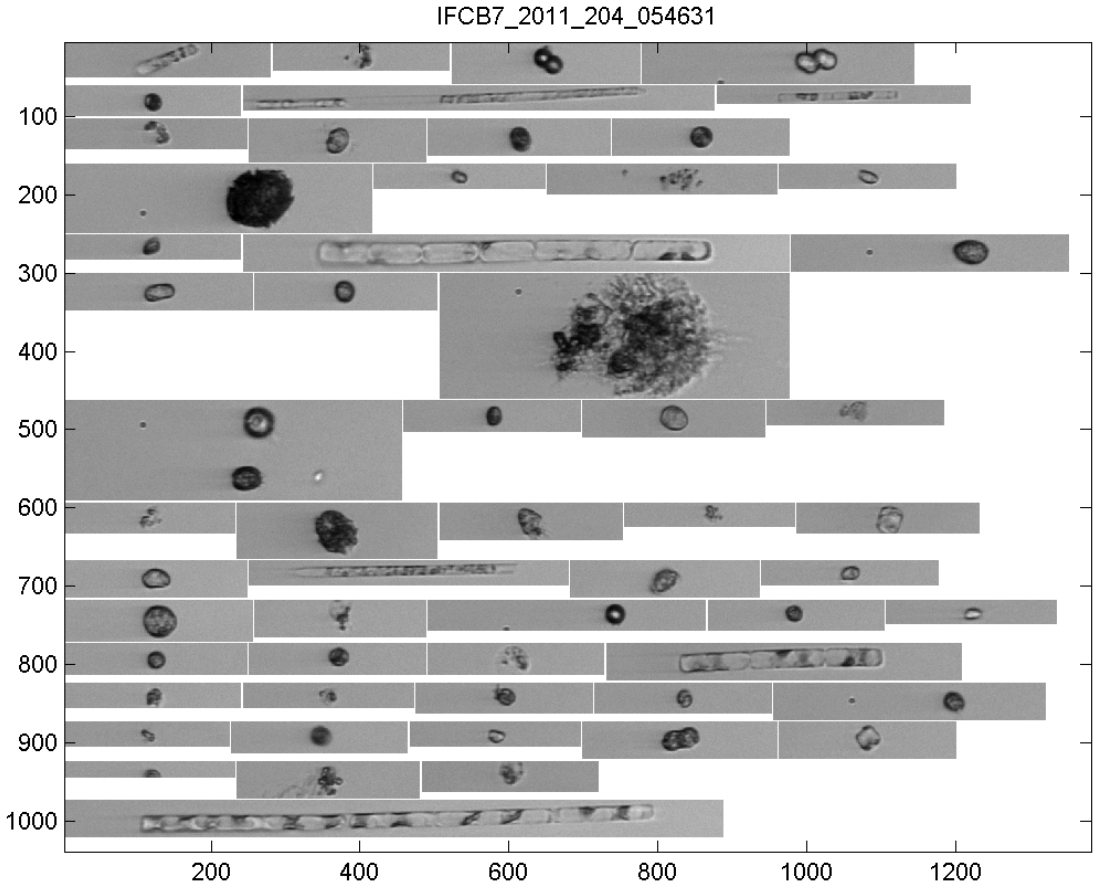

Fig. 3. Typical images from first beta unit during test deployment off WHOI dock, July 2011. Dinoflagellates and chain diatoms dominate the larger phytoplankton in July.